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THE EFFECTS OF ACID PERTURBATION ON A CONTROLLED ECOSYSTEM

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Abstract. Duplicate, 8-compartment, continuous-flow microcosms were used to study the effects of acid addition on community function, algal community structure, and degradation of a plasticizer, diethyl phthalate. Inputs of HCl decreased the alkalinity (measured as $CaCO_3$) from 25 to 8 mg l^{-1}, creating diurnal H^+ activity curves that indicated that the ecosystem was being severely stressed. Removal of excess acid was accompanied by a return to a normal diurnal pH cycle. Nutrient concentrations and O_2 production did not give a definite indication of stress resulting from the addition of acid. Algal community structure and total biomass were not affected by acid inputs. Also, degradation rates of diethyl phtalate by the aquatic bacteria were similar for the control and the acid-stressed systems. These studies indicate that acid inputs can significantly disrupt normal ecosystem function, such as diurnal pH cycling, without having a measurable impact on other parameters usually monitored in aquatic ecosystems.

1. Introduction

Combustion of fossil fuels, coal and oil in particular, can result in the formation of mineral acids (HCl, H_2SO_4, and HNO_3) in the atmosphere (Gorham, 1976) that produce acid precipitation sometimes many miles from the source. Decreases in H^+ activity (pH) in precipitation, and conversely an increase in the acidity problem, have been observed for the last several decades in Europe as well as North America (Oden, 1976). Centuries-old statues lose their features, tombstones become illegible, automobile finishes corrode, and house paints last only a few years. Equally dramatic effects of acid precipitation have been clearly observed in lakes or streams (Acid Rain, 1980), where low pH can kill the eggs of fish, salamanders, and frogs. On the other hand, no data are available that show a direct adverse effect of acid precipitation on human health. Moreover, Brock (1973) suggested that acidification can be used advantageously to control or eliminate blue-green algal blooms in lakes or streams.

The extent to which acid precipitation influences the pH of a natural water is largely dependent upon the buffering capacity of that water. Alkalinity is in part a function of the total carbonate species (CO_2 + HCO_3^- + CO_3^{-2}) which are excellent buffering agents. At a pH above 7 the relation tends towards the HCO_3^- and CO_3^{-2} species, and aquatic systems will not be affected as much by acid inputs. At a pH below 7 a shift occurs towards CO_2 which decreases the alkalinity and buffering capacity. This leads to a less stable system, and rapid changes may occur in the biota behavior.

The objectives of the experiment were to study the effects of acid addition on the chemistry or nutrients, the standing crop of the microbial communities, and the ability

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of the microbial communities to degrade a toxic substance such as diethyl phthalate (DEP) in a large laboratory microcosm.

2. Materials and Methods

2.1. Facility

The study was conducted in the EPA's Aquatic Ecosystem Simulator (AEcoS). The facility is a controlled environmental chamber that houses a 19.5 m long, 46 cm wide and 51 cm deep 'U-shaped' flume. The flume is divided into two independent flow channels (designated A and B), each subdivided into eight 250-l compartments called Continuously Stirred Tank Reactors (CSTRs). Each CSTR is equipped with an outlet weir such that the effluent from an upstream compartment constitutes the influent for the next downstream compartment.

The flume, constructed of Plexiglas, is lined with adhesive-backed, 4-mil Teflon film. Uniform mixing in each CSTR is accomplished by Teflon-covered Plexiglas paddlewheels that are suspended longitudinally down the channel.

2.2. Colonization

Prior to the input of diethyl phthalate, biological communities were established in CSTRs 3-8 by inoculating each CSTR with a 4-l inoculum from three local ponds and a stream. The CSTRs were reinoculated and cross inoculated (between Channels A and B) each week. We wanted to set up an ecosystem without a toxicant input to establish reference and control data. After a 24-week period of colonization, pH, DO, and nutrient levels

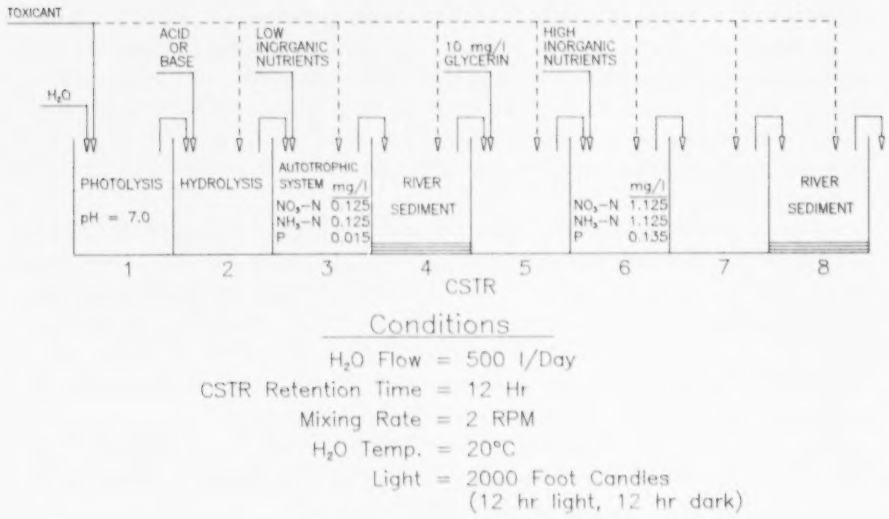

Fig. 1. AEcoS experimental setup.

idicated steady state conditions, and DEP was introduced for hydrolysis and microbial transformation studies.

2.3. Experimental design

Figure 1 shows the experimental conditions of Channels A and B, each of which was a replicate of the other. CSTR 1 received deionized and sterilized water to study the photolysis rate of the toxicant. CSTR 2 was designed to enhance the process of alkaline hydrolysis through the addition of 0.03 M NaOH. CSTR 3 was designed to enhance primary producer activity and received inorganic nutrient concentrates that were modifications of Freeman's Reference Water (Freeman, 1953) with the addition of micronutrients (Miller *et al.*, 1978). Low amounts of N and P were added (10% of total) to see whether differences in the rate of community development could be observed. CSTR 5 received a solution of glycerin to stimulate heterotrophic growth. High amounts of N and P (90% of total) were added to CSTR 6 to determine whether the rate of community development could be enhanced. Concentrations of elements after final dilution are shown in Table I.

TABLE I

Elemental concentrations in AEcoS

Macronutrients	Conc. mg l^{-1}	Micronutrients	Conc. µg l^{-1}
P	0.15	B	31.0
Si	2.94	Mn	11.1
HCO_3	34.50	Zn	14.9
Mg	1.33	Fe	31.4
K	2.77	Co	0.329
N	2.50	Cu	0.00354
Ca	4.82	Mo	2.75
Na	26.33[a]	EDTA	440
SO_4	8.66		
Cl	43.32[a]		

[a] Concentration after excess acid addition.

After colonization and upon introduction of the DEP, a pH of 10 was established in CSTR 2 for the study of alkaline hydrolysis through the addition of NaOH equivalent to 0.358 meq l^{-1}. CSTR 3 received, depending on the study, 0.252 meq HCl l^{-1}, 0.610 meq HCl l^{-1} or 0.968 meq HCl l^{-1}. The highest concentration of acid, 0.968 meq l^{-1}, included 0.252 meq l^{-1} to neutralize the sodium salts from the nutrient formula, 0.358 meq l^{-1} to neutralize the NaOH feeding into CSTR 2, and 0.358 meq l^{-1} to reduce the overall pH, cycling between 7 and 10, by one unit and to identify a possible stress effect.

2.4. Diethyl phthalate

After colonization, 191 (SD ± 6.0, N = 20) µg l^{-1} of DEP was added to each CSTR consecutively. Its fate was determined by measuring the concentration in the water

allowing 7 days for the CSTR to achieve steady state. First-order degradation rate coefficients were then calculated. DEP concentrations were determined by gas-liquid chromatography using a Tracor 550 gas-liquid chromatograph equipped with a Ni^{63} electron capture detector and a 2-m (4-mm I.D.) glass column packed with 3% SE-30 on 80/100 mesh Gas-Chrom Q. Water samples were extracted and prepared according to Paris *et al.* (1975).

2.5. Analytical Chemistry

The pH was measured automatically in each CSTR at 3-h intervals (Beckman Models 941 and 960A monitoring systems). O_2 (Orbisphere Model 2708), NO_3–N, NH_3–N, and o–P (Methods for Chemical Analysis of Water and Wastes, EPA-600/4-79-020, March 1979) were measured in the water at 0830 and 1530 in each CSTR once a week.

2.6. Analytical Biology

Periphyton populations were colonized for 4 weeks on 10 × 30-cm Teflon-covered plastic sheets suspended in each CSTR. The biomass was scraped from the sheets, blended for 5 s, and subsamples were analyzed. Chlorophyll *a*, corrected for pheophytin *a* according to Strickland and Parsons (1968), was determined using a B. Braun cell homogenizer and a G. K. Turner and Associates (Model III) fluorometer. Ash-free dry weight determinations were made using the procedure outlined for periphyton in Standard Methods (1976). Algae were enumerated using the inverted microscope method of Utermohl as described by Lund (1958).

2.7. Data

The raw data of all parameters were logged into a computer for the computation of finished data and the construction of graphs for data evaluations. Statistical evaluations were performed using Student's t-test and analysis of variance.

3. Results

All CSTRs exhibited regular diurnal pH patterns during the 6-mo colonization period when acid inputs were 0.252 meq l^{-1} (Figure 2 top). Replicate CSTRs, in this case CSTR 3, nearly always had identical pH cycles.

When DEP was added to the channels, 0.358 meq NaOH l^{-1} was added to CSTR 2, to elevate the pH to 10. HCl was added to CSTR 3 in excess (0.968 meq l^{-1}) to neutralize the NaOH carried over from CSTR 2 and to lower the average pH of the system for stress analysis. Upon addition of the acid, the diurnal pH cycle was interrupted (Figure 2 middle) and did not stabilize during a 10-week input period. This addition of acid resulted in systems from CSTR 3 through 8 having average pH values between 4 and 5, as opposed to 7 and 10 before the addition (Table II).

Decreasing the acid from 0.968 meq l^{-1} to 0.252 meq l^{-1} and also eliminating the 0.358 meq NaOH l^{-1} in CSTR 2, immediately produced a normal diurnal pH cycle. This stable cycle was maintained for an 8-week test period.

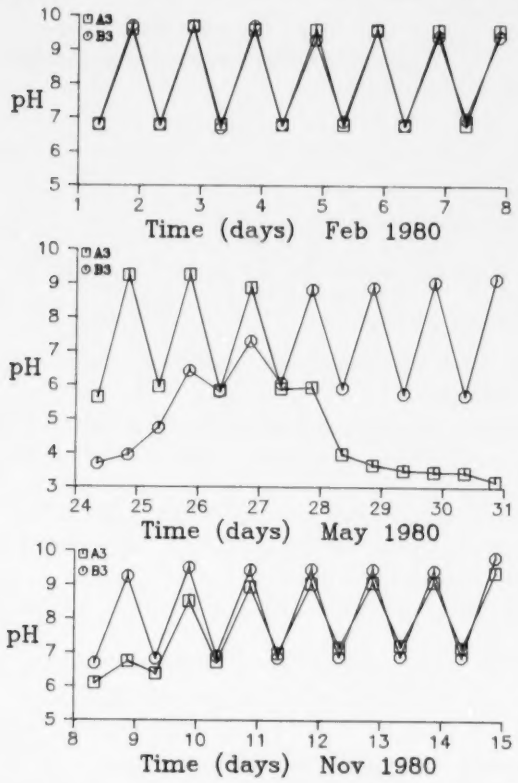

Fig. 2. Acid perturbation. (top) using 0.252 meq HCl/0.252 meq NaOH; (middle) using 0.968 meq HCl/0.610 meq NaOH; (bottom) using 0.610 meq HCl/0.610 meq NaOH. A3 = channel A, CSTR 3; B3 = channel B, CSTR 3.

When 0.968 meq HCl l^{-1} was again added to both channels (including 0.358 meq NaOH l^{-1} to CSTR 2), the non-coherent pH pattern developed as before. When the acid input was decreased to 0.610 meq l^{-1} (Figure 2 bottom), eliminating the excess acid, however, the diurnal pH cycle returned to a pattern similar to that of the

TABLE II

pH values in AEcoS before and after addition of acid. Ranges around the mean are in parentheses.

	A (morning)	B (morning)	A (evening)	B (evening)
Average pH before acid addition (range)	7.01 (6.48–7.90)	7.26 (6.92–8.00)	9.63 (9.68–10.10)	10.00
Average pH during acid addition (range)	4.67 (3.12–9.26)	5.10 (3.68–9.44)	4.69 (3.18–9.38)	5.08 (3.94–10.10)

TABLE III

Acid/base conditions and corresponding alkalinities

	Base, meq l^{-1}	Acid, meq l^{-1}	Alkalinity, mg $CaCO_3$ l^{-1}
Colonization	0.252	0.252[a]	25.0
Hydrolysis	0.610[b]	0.610	23.4
pH adjustment during hydrolysis	0.610	0.968[c]	7.9

[a] Acid required to neutralize Na salts in nutrient formula to pH 7.5
[b] 0.358 meq l^{-1} NaOH added to CSTR 2.
[c] 0.358 meq l^{-1} HCl added to CSTR 3 for pH adjustment.

colonization time. Table III illustrates the three conditions of acid inputs and their equivalent base additions.

Other measurements of ecosystem function did not provide a definite indication of stress due to the acid addition. For example, nutrient concentrations calculated for nitrate showed no difference between normal and acid-perturbed systems. A short-term increase in the export of ortho phosphate and ammonia was noted only in Channel A during the first perturbation period. No additional export occurred during the second time both channels received 0.968 meq l^{-1}.

The diurnal O_2 curves were also similar for systems receiving both 0.252 and 0.968 meq HCl l^{-1}.

Total biomass was not significantly different for normal and acid-perturbed systems in terms of ash-free dry weight and chlorophyll *a* (using Student's *t*-test at an Alpha Level of 0.05). An analysis of variance of the algal data indicated that there was no difference between the normal and acid-perturbed systems (Alpha Level = 0.05).

An analysis of variance test was performed to compare first-order rate coefficients (k_1) of acid versus non-acid perturbed systems. Data from CSTRs 4, 5, and 6 were used for this evaluation. Four treatments were used for the statistical analysis:

1. Channel A with acid;
2. Channel A without acid (7 weeks later);
3. Channel B without acid;
4. Channel B without acid (7 weeks later).

No differences were observed between the four treatments when the following contrasts were made at an Alpha Level of 0.05.

1. Channel A with acid versus Channel A without acid;
2. Channel A with acid versus Channel B without acid;
3. Channel B without acid versus Channel B without acid.

4. Discussion

The non-coherent pH pattern can be attributed to a loss of buffering capacity or alkalinity. Alkalinity is defined by Stumm and Morgan (1970) as the capacity of a system to neutralize strong acids. They give an 'average river' alkalinity of 48 mg l^{-1} as $CaCO_3$.

Glass *et al.* (1979) give values for some lakes in the Adirondacks of 35 to 45 mg l^{-1} as $CaCO_3$. They suggest that lakes with an alkalinity below 30 mg l^{-1} are potentially susceptible to acid stress and below 15 mg l^{-1} are severely affected by acid conditions. Alkalinity in the AEcoS averaged 25 mg l^{-1} as $CaCO_3$ when 0.252 meq acid l^{-1} was added during colonization, and it decreased to 7.9 mg l^{-1} with the addition of 0.968 meq acid l^{-1}. Increased acid inputs decreased the alkalinity and increased the system's susceptibility to become highly stressed. These results confirm the generalization made by Glass *et al.* (1979).

As more acid was added to the system, a decrease in buffering capacity occurred. The low pH, then, caused the neutralization of all carbonate species to a high concentration of CO_2 in the water. Any influence by atmospheric CO_2 on the system was negligible since the CO_2 concentration in the water was much higher than the normal equilibrium concentration between distilled water and atmospheric CO_2.

Natural aquatic systems have buffering reservoirs due to sediments, weak organic acids, and the diversity of chemical inputs (ground water inflow, rain, dry deposition, etc.). Grahn *et al.* (1974) suggested that in acid systems in Sweden, sulfate replaced carbonate as the major anion. Schindler *et al.* (1980) concluded that, in the experimental Lakes Area, dissolved inorganic carbon respired by decomposers buffered against acidificatio- AEcoS was limited in its buffering capacity, a condition that perhaps should be remedied.

No change was observed in the concentrations of nitrate and oxygen. A rapid short-term increase (beyond the added concentration) was noted in the concentrations of ammonia and ortho phosphate in Channel A, but not in Channel B. This short-term increase was not observed during the second time of excess acid perturbation. Hall *et al.* (1980) found no change in the concentrations of nitrate and ammonia between acidified and non-acidified systems, whereas Schindler *et al.* (1980) found a substantial increase in the concentration of ammonia during the period of acidification. In terrestrial systems, hydrogen displaces other cations from adsorption sites on soil particles. If this process were also to affect adsorbed nutrients on both living organisms and particulate organic matter, it could account for the release of ammonia. A hydrolysis effect could account for the increase in ortho phosphate. It is possible that the short-term low pH was responsible for the increase due to hydrolysis to ortho phosphate.

No difference was noted in the biota in terms of chlorophyll *a*, ash-free dry weight, and algal count. Several researchers (Hall *et al.*, 1980; Müller, 1980; Hendrey, 1976) found increases in the periphyton populations when systems were acidified to pH 4. Additionally, Hall *et al.* (1980) found a significant increase in chlorphyll *a*, whereas Schindler *et al.* (1980) found no great difference in the average concentration of chlorophyll *a* during the period of acidification. Schindler and coworkers acidified the lake water to a pH of 5.7 to 5.9 in contrast to other researchers who acidified to a pH of 4. Kwiatkowski *et al.* (1976) studied lakes with a neutral pH between 4.05 and 7.15 and discovered that chlorophyll *a* concentrations of phytoplankton decreased with declining pH. Müller (1980) found a significant change in the composition of the three main algae groups (Cyanophyta, Chlorophyta, and Chrysophyta). Diatomae were dominant between

pH 6.25 and 6.5 and decreased in biomass with decreasing pH. He concluded that a different composition of algal species was the reason for the increase in periphyton growth at the acid pH. Almer *et al.* (1974) also found that most of the diatom species disappeared below pH 5.8. Brock (1973) found that Cyanophyta were completely absent from habitats below pH 4 to 5. Kwiatkowski *et al.* (1976) discovered that Chlorophyta diminished in importance at the lower pH and Cyanophyta became dominant. Primary production was reduced below pH 5.5, but increased as the pH approached neutrality. Our findings on algal structure with respect to acid perturbation indicate that the structure of the community was unaffected by the addition of the excess acid to the system. The three channel combinations were tested for differences in k_1 for DEP degradation. None of these contrasts showed significant differences, indicating that there were no changes in DEP rates during the experiment.

5. Conclusions

From our findings, which are summarized in Table IV, we conclude that the cause of the observations we have made was purely chemical. Acid perturbation below a pH of 6 can severely stress an ecosystem to the point of losing its buffering capacity with little or no effect on community function and does not seem to effect uptake or release of major nutrients by microorganisms. The effects of acid addition on algal community structure and total biomass were not significant. The biodegradation rate of the plasticizer diethyl phthalate was not affected by acid perturbation. Additional research needs to be done to investigate the transition point in decreasing pH at which population and dominance (and perhaps adaptation) changes do occur that will ultimately have an effect on a vital process such as biodegradation of toxicants.

TABLE IV

Summary of parameters measured in AEcoS and observed responses during acid perturbation

Parameter	CSTR A3	CSTR B3
pH	slow decrease	rapid decrease to 3.9
	long period pH 4	one day pH 4
	Both A and B showed incoherent pattern	
	Both sides returned to equilibrium after acid removed	
alkalinity	Both sides decreased to below 10 mg l^{-1} $CaCO_3$	
NH_3–N	rapid, short-term release	no observable change
NO_3–N	no observable change	no observable change
o–P	rapid, short-term release	no observable change
Oxygen	no observable change	no observable change
Chlorophyll *a*	no change	no change
Total algal counts	no change	no change
Algal structure	no change	no change
Ash-free dry weight	no change	no change
DEP degradation	no change	no change

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